## STATE OF MISSOURI

## DEPARTMENT OF NATURAL RESOURCES

## MISSOURI CLEAN WATER COMMISSION



# MISSOURI STATE OPERATING PERMIT

In compliance with the Missouri Clean Water Law, (Chapter 644 R.S. Mo. as amended, hereinafter, the Law), and the Federal Water Pollution Control Act (Public Law 92-500, 92<sup>nd</sup> Congress) as amended,

Permit No. MO-0121827

Owner: Associated Electric Cooperative, Inc. (AECI)

Address: P.O. Box 754, Springfield, MO 65801

Continuing Authority: Same as above Address: Same as above

Facility Name: AECI, St. Francis Power Plant

Facility Address: Rt. 1, Box 441, Campbell, MO 63933

Legal Description: NE ¼, E ½, Sec. 3, T22N, R8W, Dunklin County

Latitude/Longitude: See Page Two

Receiving Stream: St. Francis River(P)

First Classified Stream and ID: St. Francis River (P)(02968)

USGS Basin & Sub-watershed No.: (08020203-030002)

is authorized to discharge from the facility described herein, in accordance with the effluent limitations and monitoring requirements as set forth herein:

## **FACILITY DESCRIPTION**

See Page Two

This permit authorizes only wastewater discharges under the Missouri Clean Water Law and the National Pollutant Discharge Elimination System; it does not apply to other regulated areas. This permit may be appealed in accordance with Section 644.051.6 of the Law.

December 6, 2000 July 25, 2003
Effective Date Revised

Stephen M Mahfor I, Director Department of Natural Resources Executive Secretar, Clean Water Commission

December 5, 2005

Expiration Date MO 780-0041 (10-93) Jim Hull, Director of Staff, Clean Water Commission

## FACILITY DESCRIPTION (continued)

## Outfall #001 - SIC #4911

Discharge of the cooling tower blowdown from unit 1 at a design flow rate of approximately 0.467 MGD. Latitude: +3635114 Longitude: -09010434

## Outfall #002 - SIC #4911

Discharge from the oil water separator from unit 1 at a design flow rate of  $0.360~\mathrm{MGD}$ , which represents part of the total categorical low volume waste discharge from this unit. Latitude:  $+3635114~\mathrm{Longitude}$ :  $-09010434~\mathrm{Longitude}$ :

#### Outfall #003 - SIC #4911

Discharge from the cooling tower pond which contains the total flow from Outfalls #001 and #002 that serves unit 1 plus the backwash water from the non "ion exchange" water treatment process at a flow rate of approximately 0.840 MGD. This outfall will be the sampling point for pollutants of water quality concern. Latitude: +3635078 Longitude: -09010478

#### Outfall #004 - SIC #4911

Discharge point from the storm water collection basin from the entire power plant complex. Discharge will be dependent upon rainfall. Latitude: +3635114 Longitude: -09010434

## Outfall #005 - SIC #4911

Discharge of cooling tower blow down from unit 2 at a design flow rate of approximately 0.467 MGD. Latitude: +3635059 Longitude: -09010356

#### Outfall #006 - SIC #4911

Discharge from the oil water separator from unit 2 at a design flow rate of 0.360~MGD, which represents part of the total categorical low volume waste discharge from this unit. Latitude: +3635059~Longitude: -09010356

## Outfall #007 - SIC #4911

Discharge from the cooling tower pond which contains the total flow from outfalls #005 and #006 that serves unit 2. Latitude: +3635059 Longitude: -09010356

All outfalls combine and discharge into a closed oxbow, which flows via a surface and subsurface route to an open oxbow of the St. Francis River. This discharge point is located in the NE ¼, E ½, Sec. 3, T22N, R8E, of Dunklin County, Missouri.

## A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

PAGE NUMBER 3 of 12

PERMIT NUMBER MO-0121827

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

		FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #001 - Cooling Tower B	low down	- Unit 1				
Flow	MGD	*		*	daily	24 hr. estimate
Temperature Chromium, Total	°F	*		*	daily	grab
(Special Condition 12)	mg/L	0.2		0.2	once/month	grab
Zinc, Total (Special Condition 12)	mg/L	1.0		1.0	once/month	grab
pH Units	SU	**		* *	once/month	grab
126 Priority Pollutants*****	mg/L	No Detection (Special Condition 9)		once/month	grab	
MONITORING REPORTS SHALL BE SUI	BMITTED MO	ONTHLY; TH	E FIRST REI	PORT IS DU	E January 28, 2001.	
Outfall #002 - Oil Water Separ	ator - Un	it 1				
Flow	MGD	*		*	once/month	24 hr. estimate
Oil and Grease	mg/L	20		15	once/month	grab
pH Units	SU	**		* *	once/month	grab
MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE January 28, 2001.						

## A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

PAGE NUMBER 4 of 12

PERMIT NUMBER MO-0121827

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

		FINAL EF	FLUENT LIM	TATIONS	MONITORING REQUIREMENTS		
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE	
Outfall #003 - Cooling Water Pond - Unit 1							
Flow	MGD	*		*	once/month	24 hr. estimate	
pH - Units	SU	****		****	once/month	grab	
Total Suspended Solids	mg/L	100		30	once/month	grab	
Oil & Grease	mg/L	15		10	once/month	grab	
Chromium, Total (Special Condition 12)	mg/L	0.2		0.2	once/month	grab	
Zinc, Total (Special Condition 12)	mg/L	1.0		1.0	once/month	grab	
Free Available Chlorine****	mg/L	0.5		0.2	once/month	grab	
Temperature (Special Condition 13)	°F	95		95	once/month	grab	
126 Priority Pollutants*****	mg/L	No Detection (Special Condition 9)			once/month	grab	
MONITORING REPORTS SHALL BE SUBM	MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE January 28, 2001.						
Whole Effluent Toxicity (WET) Test	% Survival	(See Special Condition 10)		Once/year	24 hr. composite		
MONITORING REPORTS SHALL BE SUBMITTED ONCE EVERY 5 YEARS; THE FIRST REPORT IS DUE October 28, 2001.							

## A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS

PAGE NUMBER 5 of 12

PERMIT NUMBER MO-0121827

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

		FINAL EFFLUENT LIMITATIONS			MONITORING REQUIREMENTS	
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE
Outfall #004 - Storm water						_
Flow	MGD	*		*	once/year	24 hr. estimate
pH - Units	SU	* *		**	once/year	grab
Settleable Solids	mL/L/hr	2.5		1.5	once/year	grab
Oil and Grease	mg/L	15		10	once/year	grab
Total Residual Chlorine (Note 1)	mg/L	*		*	once/year	grab
MONITORING REPORTS SHALL BE S			THE FIRST	REPORT I	S DUE October 28,	<u>2001.</u>
Outfall #005 - Cooling Tower	1	_	1	1 .	1	
Flow	MGD	*		*	daily	24 hr. estimate
	SU	* *		**	once/month	grab
pH Units	°F	*		*	daily	grab
Temperature	mg/L	0.2		0.2	once/month	grab
Chromium Total (Special Condition 12)	mg/L	1.0		1.0	once/month	grab
Zinc, Total (Special Condition 12)	mg/L	No De	etection -	- (See	once/month	grab
126 Priority Pollutants****		Speci	al Condit	ion 9)		
MONITORING REPORTS SHALL BE SUBM	I IITTED <u>MONT</u>	<u> </u>	IRST REPOR	T IS DUE J	lanuary 28, 2001.	
Outfall #006 - Oil Water Sep	parator - I	Jnit 2				
Flow	MGD	*		*	once/month	24 hr. estimate
pH Units	SU	**		**	once/month	grab
Oil and Grease	mg/L	20		15	once/month	grab
MONITORING REPORTS SHALL BE SUBM	I MITTED MONT	<u> </u>	RST REPOR	T IS DUF Jai	L nuary 28 2001	

MO 780-0010 (8/91)

					PAGE NUMBER	6 of 12
A. EFFLUENT LIMITATIONS AND I	PERMIT NUMBER MO-0121827					
		FINAL EFFLUENT LIMITATIONS			MONITORING RE	EQUIREMENTS
OUTFALL NUMBER AND EFFLUENT PARAMETER(S)	UNITS	DAILY MAXIMUM	WEEKLY AVERAGE	MONTHLY AVERAGE	MEASUREMENT FREQUENCY	SAMPLE TYPE

The permittee is authorized to discharge from outfall(s) with serial number(s) as specified in the application for this permit. The final effluent limitations shall become effective upon issuance and remain in effect until expiration of the permit. Such discharges shall be controlled, limited and monitored by the permittee as specified below:

Outfall #007 - Cooling Water Pond - Unit 2

Flow	MGD	*		*	once/month	24 hr. estimate
pH - Units	SU	****		****	once/month	grab
Total Suspended Solids	mg/L	100		30	once/month	grab
Oil & Grease	mg/L	15		10	once/month	grab
Chromium, Total (Special Condition 12)	mg/L	0.2		0.2	once/month	grab
Zinc, Total (Special Condition 12)	mg/L	1.0		1.0	once/month	grab
Free Available Chlorine***	mg/L	0.5		0.2	once/month	grab
Temperature (See Special Condition 13)	°F	95		95	once/month	grab
126 Priority Pollutants*****	mg/L		ction See ondition S		once/month	grab

MONITORING REPORTS SHALL BE SUBMITTED MONTHLY; THE FIRST REPORT IS DUE January 28, 2001.

Whole Effluent Toxicity (WET) Test	% Survival	(See Special Condition 10)	once/year	24 hr. composite

MONITORING REPORTS SHALL BE SUBMITTED <u>ANNUALLY</u>; THE FIRST REPORT IS DUE <u>October 28, 2001.</u> THERE SHALL BE NO DISCHARGE OF FLOATING SOLIDS OR VISIBLE FOAM IN OTHER THAN TRACE AMOUNTS.

## **B. STANDARD CONDITIONS**

IN ADDITION TO SPECIFIED CONDITIONS STATED HEREIN, THIS PERMIT IS SUBJECT TO THE ATTACHED <u>Part I</u> STANDARD CONDITIONS DATED <u>October 1, 1980</u>, AND HEREBY INCORPORATED AS THOUGH FULLY SET FORTH HEREIN.

MO 780-0010 (8/91)

## A. EFFLUENT LIMITATIONS AND MONITORING REQUIREMENTS (continued)

- \* Monitoring requirement only.
- \*\*pH is measured in pH units and is not to be averaged. The pH is limited to the range of 6.0-9.0 pH units.
- \*\*\* Sample once per quarter in the months of March, June, September, December.
- \*\*\*\* Free Available Chlorine is defined in 40 CFR 423.11.
- \*\*\*\*\*pH is measured in pH units and is not to be averaged. The pH is limited to the range of 6.0 10.5 pH units.
- \*\*\*\*\*\*Only those pollutants contained in chemicals added for cooling tower maintenance, except Chromium, Total and Zinc, Total, which will be limited as shown. See Special Condition #10.

Note 1 - This permit contains a Total Residual Chlorine (TRC) limit.

a. If the TRC limit in this permit is 0.01 mg/L or 0.2 mg/L, you <u>must use</u> an analytical method that has a quantification limit of no greater than 0.05 mg/L TRC. For reporting purposes on the discharge monitoring report (DMR), all analytical values below 0.05 mg/L shall be reported as "<quantlim." All analytical values at or above the quantification limit of 0.05 mg/L shall be reported as the measured value. The permittee shall report the quantification limit in the remarks section of the DMR.

The average monthly effluent values for TRC will be determined by assuming that analytical results below the quantification limit are equivalent to 0 mg/L when calculating the monthly average.

The daily effluent value will be considered equal to 0 mg/L if it is below the quantification limit.

b. If the TRC limit in this permit is 1.0 mg/L; you <u>must use</u> an analytical method with a quantification limit between 0.2 and 0.5 mg/L. All analytical values below the quantification limit shall be reported as "<quantlim." All analytical values at or above the quantification limit shall be reported as the measured value.

The average monthly effluent values for TRC will be determined by assuming that analytical results below the quantification limit are equivalent to 0 mg/L when calculating the monthly average.

The daily effluent value will be considered equal to 0  $\mbox{mg/L}$  if it is below the quantification limit.

- c. Disinfection is required year-round unless the permit specifically states that "Final limitations and monitoring requirements for Fecal Coliform are applicable only during the recreational season from April 1 through October 31." If your permit does not require disinfection during the non-recreational months, do not chlorinate in those months.
- d. Do not chemically dechlorinate if it is not required in your permit.
- e. If no chlorine was used in a given sampling period, an actual analysis is not necessary. Simply report as "0 mg/L" TRC.

## C. SPECIAL CONDITIONS

- 1. There shall be no discharge of polychlorinated biphenyl compounds such as those commonly used for transformer fluid.
- 2. This permit may be reopened and modified, or alternatively revoked and reissued, to:
  - (a) Comply with any applicable effluent standard or limitation issued or approved under Sections 301(b)(2)(C) and (D), 304(b)(2), and 307(a) (2) of the Clean Water Act, if the effluent standard or limitation so issued or approved:
    - (1) contains different conditions or is otherwise more stringent than any effluent limitation in the permit; or
    - (2) controls any pollutant not limited in the permit.
  - (b) Incorporate new or modified effluent limitations or other conditions, if the result of a waste load allocation study, toxicity test or other information indicates changes are necessary to assure compliance with Missouri's Water Quality Standards.
  - (c) Incorporate new or modified effluent limitations or other conditions if, as the result of a watershed analysis, a Total Maximum Daily Load (TMDL) limitation is developed for the receiving waters which are currently included in Missouri's list of waters of the state not fully achieving the state's water quality standards, also called the 303(d) list.

The permit as modified or reissued under this paragraph shall also contain any other requirements of the Clean Water Act then applicable.

- 3. All outfalls must be clearly marked in the field.
- 4. Report as no-discharge when a discharge does not occur during the report period.
- 5. General Criteria. The following water quality criteria shall be applicable to all waters of the state at all times including mixing zones. No water contaminant, by itself or in combination with other substances, shall prevent the waters of the state from meeting the following conditions:
  - (a) Waters shall be free from substances in sufficient amounts to cause the formation of putrescent, unsightly or harmful bottom deposits or prevent full maintenance of beneficial uses;
  - (b) Waters shall be free from oil, scum and floating debris in sufficient amounts to be unsightly or prevent full maintenance of beneficial uses;
  - (c) Waters shall be free from substances in sufficient amounts to cause unsightly color or turbidity, offensive odor or prevent full maintenance of beneficial uses;
  - (d) Waters shall be free from substances or conditions in sufficient amounts to result in toxicity to human, animal or aquatic life;
  - (e) There shall be no significant human health hazard from incidental contact with the water;
  - (f) There shall be no acute toxicity to livestock or wildlife watering;
  - (g) Waters shall be free from physical, chemical or hydrologic changes that would impair the natural biological community;
  - (h) Waters shall be free from used tires, car bodies, appliances, demolition debris, used vehicles or equipment and solid waste as defined in Missouri's Solid Waste Law, 6. Section 260.200, RSMo, except as the use of such materials is specifically permitted pursuant to section 260.200-260.247.
- 6. An annual report shall be prepared and submitted to the Missouri Department of Natural Resources concerning the compressor turbine cleaning wastewater. Dates of cleaning, volume of wastewater generated, the analysis of the wastewater for each unit shall be included in the report along with the location of the planned disposal site.

## C. SPECIAL CONDITIONS

- 7. The permittee shall collect and have analyzed a sample from Outfall #003 and Outfall #004 as defined in this permit. This analysis shall be for all the pollutants on Part A and those marked as "believed present" on Part B of the permit application Form C, and for all the pollutants marked as "testing required" on the permit application Form D. This analysis shall be submitted to the Missouri Department of Natural Resources within six months of start up of the power plant.
- 8. Any sludge that is removed from the cooling water basin shall be analyzed for TCLP. The MDNR shall be supplied a copy of the results along with a disposal plan for review and approval.
- 9. Instead of monitoring, compliance with the limitations for the 126 priority pollutants may be determined by engineering calculations which demonstrate that the regulated pollutants are not detectable in the final discharge of Outfall #001 by the analytical methods in 40 CFR Part 136. This is allowed in Part 40 CFR 423.15(J)(3).
- 10. Whole Effluent Toxicity (WET) tests will be conducted as follows:

MONITORING REPORTS SHALL BE SUBMITTED AS SPECIFIED IN THE TABLE BELOW, THE FIRST REPORT IS DUE October 28, 2001.

SUMMARY OF WET TESTING FOR THIS PERMIT								
OUTFALL	A.E.C. %	FREQUENCY	SAMPLE TYPE	MONTH				
#003	100%	Annually	24 hr. composite	September				
#007	100%	Annually	24 hr. composite	September				

- a. Test Schedule and Follow-Up Requirements
  - (1) Perform a single-dilution test in the months and at the frequency specified above.

If the test passes the effluent limit do not repeat test until the next test period. Submit results with the annual report.

If the test fails the effluent limit a multiple dilution test shall be performed within 30 days, and biweekly thereafter until one of the following conditions are met:

- (a) THREE CONSECUTIVE MULTIPLE-DILUTION TESTS PASS. No further tests need to be performed until next regularly scheduled test period.
- (b) A TOTAL OF THREE MULTIPLE-DILUTION TESTS FAIL.
- (2) The permittee shall submit a summary of all test results for the test series to the Planning Section of the WPCP, DNR, Box 176, Jefferson City, MO within 14 days of the third failed test. DNR will contact the permittee with initial guidance on conducting a toxicity identification evaluation (TIE) or toxicity reduction evaluation (TRE). The permittee shall submit a plan for conducting a TIE or TRE to the Planning Section of the WPCP within 60 days of the date of DNR's letter. This plan must be approved by DNR before the TIE or TRE is begun. A schedule for completing the TIE or TRE shall be established in the plan approval.
- (3) Upon DNR's approval, the TIE/TRE schedule may be modified if toxicity is intermittent during the TIE/TRE investigations. A revised WET test schedule may be established by DNR for this period.

Page 10 of 12 Permit No. MO-0121827

## C. SPECIAL CONDITIONS (continued)

- 10. Whole Effluent Toxicity (WET) tests (continued)
  - a. Test Schedule and Follow-Up Requirements (continued)
    - (4) If a previously completed TIE has clearly identified the cause of toxicity, additional TIEs will not be required as long as effluent characteristics remain essentially unchanged and the permittee is proceeding according to a DNR approved schedule to complete a TRE and reduce toxicity. Regularly scheduled WET testing as required in part b.(1) will be required during this period.
    - (5) In addition to the WET test summary report required in part (2), all failing test results shall be reported to DNR within 14 days of the availability of results.
    - (6) All WET test results for the reporting period shall be summarized and submitted to DNR by the end of the following October. When WET test sampling is required to run over one DMR period, each DMR report shall contain information generated during the reporting period.
  - b. PASS/FAIL procedure and effluent limitations
    - (1) To pass a single-dilution test, mortality observed in the AEC test concentration shall not be significantly different (at the 95% confidence level; p = 0.05) than that observed in the upstream receiving-water control. The appropriate statistical tests of significance will be those outlined in the most current USEPA acute toxicity manual or those specified by the MDNR.
    - (2) To pass a multiple-dilution test:
      - (a) the computed percent effluent at the edge of the zone of initial dilution (AEC) must be less than three-tenths (0.3) of the  $LC_{50}$  concentration for the most sensitive of the test organisms, or,
      - (b) all dilutions equal to or greater than the AEC must be nontoxic. Failure of one multiple-dilution test is considered an effluent limit violation.

## c. Test Conditions

- (1) Test species: Ceriodaphnia dubia and fathead minnows, Pimephales promelas. Organisms used in WET testing should come from cultures reared for the purpose of conducting toxicity tests and should be cultured in a manner consistent with the most current USEPA guidelines. All test animals should be cultured as described in EPA-600/4-90/027.
- (2) Test period: 48 hours at the "Acceptable Effluent Concentration" (AEC) specified above.
- (3) When dilutions are required, upstream receiving stream water will be used as dilution water. If upstream water is unavailable or if mortality in the upstream water exceeds 10%, "reconstituted" water will be used. Procedures for generating reconstituted water will be supplied by the Department of Natural Resources (DNR).
- (4) Tests should be initiated immediately after the sample is collected, but tests must be initiated no later than 36 hours after collection.

## C. SPECIAL CONDITIONS (continued)

- 10. Whole Effluent Toxicity (WET) tests (continued)
  - c. Test Conditions (continued)
    - (5) Single-dilution tests will be run with:
      - (a) Effluent at the AEC concentration;
      - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
      - (c) reconstituted water.
    - (6) Multiple-dilution tests will be run with:
      - (a) 100%, 50%, 25%, 12.5%, and 6.25% effluent, unless the AEC is less than 25% effluent, in which case dilutions will be 4 times the AEC, two times the AEC, AEC, ½ AEC and ¼ AEC.
      - (b) 100% receiving-stream water (if available), collected upstream of the outfall at a point beyond any influence of the effluent; and
      - (c) reconstituted water.
    - (7) If reconstituted-water control mortality for a test species exceeds 10%, the entire test will be rerun.
- 11. MDNR must be notified each time wastewater is hauled offsite for treatment or disposal.
- 12. If permittee can demonstrate that Zinc and Chromium are not used in cooling tower maintenance chemicals, monitoring is not required. DMR should state "no detect" for these two (2) metals if they are not used in cooling tower chemicals.
- 13. Temperature will be limited to 95 degrees farenheit at outfalls 003 and 007 which discharge to the closed oxbow. Associated Electric will prepare a sampling plan for review and approval to the Department within two months of issuance of the permit. Upon approval of the sampling plan, Associated Electric will implement the plan and develop a water quality impact study report to be reviewed during the permit renewal process on both oxbows and the St. Francis River concerning at a minimum the following parameters: temperature, chlorine, copper, selenium, and flouride.

## D. SCHEDULE OF COMPLIANCE

1. The permittee shall submit a study plan for department approval which will document discharges from outfalls 003 and 007 are not creating Ph conditions in the receiving stream which exceed the status water quality standards. A study plan shall be submitted by January 1, 2001.

#### SUMMARY OF TEST METHODOLOGY FOR WHOLE-EFFLUENT TOXICITY TESTS

Whole-effluent-toxicity test required in NPDES permits shall use the following test conditions when performing single or multiple dilution methods. Any future changes in methodology will be supplied to the permittee by the Missouri Department of Natural Resources (MDNR). Unless otherwise specified by MDNR, procedures should be consistent with Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, EPA/600/4-90/027.

## Test conditions for <a href="Ceriodaphnia dubia">Ceriodaphnia dubia</a>:

Test duration: 48 h Temperature: 25  $\pm$  2°C

Light Quality: Ambient laboratory illumination

Photoperiod: 16 h light, 8 h dark Size of test vessel: 30 mL (minimum) Volume of test solution: 15 mL (minimum)

Age of test organisms: <24 h old

No. of animals/test vessel: 5
No. of replicates/concentration: 4

No. of organisms/concentration: 20 (minimum)

Feeding regime: None (feed prior to test)

Aeration: None

Dilution water: Upstream receiving water; if no upstream

flow, synthetic water modified to reflect

effluent hardness.

Endpoint: Mortality (Statistically significant

difference from upstream receiving water

control at  $p \le 0.05$ )

Test acceptability criterion: 90% or greater survival in controls

## Test conditions for (Pimephales promelas):

Test duration: 48 h Temperature: 25  $\pm$  2°C

Light Quality: Ambient laboratory illumination

Photoperiod: 16 h light/ 8 h dark Size of test vessel: 250 mL (minimum)
Volume of test solution: 200 mL (minimum)

Age of test organisms: 1-14 days (all same age)

No. of animals/test vessel: 1

No. of replicates/concentration: 4 (minimum) single dilution method

No. of organisms/concentration: 2 (minimum) multiple dilution method 40 (minimum) single dilution method

20 (minimum) multiple dilution method

Feeding regime: None (feed prior to test)

Aeration: None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min.

Dilution water:

Upstream receiving water; if no upstream flow, synthetic water modified to reflect

effluent hardness.

Endpoint: Mortality (Statistically significant

difference from upstream receiving water

control at  $p \le 0.05$ )

Test Acceptability criterion: 90% or greater survival in controls